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PharmaCool Study Grp

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# Population Pharmacokinetics of Amoxicillin in Term Neonates Undergoing Moderate Hypothermia

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The pharmacokinetics (PK) of amoxicillin in asphyxiated newborns undergoing moderate hypothermia were quantified using prospective data ( $N = 125$ ). The population PK was described by a 2-compartment model with *a priori* birthweight (BW) based allometric scaling. Significant correlations were observed between clearance (Cl) and postnatal age (PNA), gestational age (GA), body temperature (TEMP), and urine output (UO). For a typical patient with GA 40 weeks, BW 3,000 g, 2 days PNA (i.e., TEMP 33.5°C), and normal UO, Cl was 0.26 L/h (interindividual variability (IIV) 41.9%) and volume of distribution of the central compartment was 0.34 L/kg (IIV of 114.6%). For this patient, Cl increased to 0.41 L/h at PNA 5 days and TEMP 37.0°C. The respective contributions of both covariates were 23% and 27%. Based on Monte Carlo simulations we recommend 50 and 75 mg/kg/24h amoxicillin in three doses for patients with GA 36–37 and 38–42 weeks, respectively.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ To date, there are no data on the effect of moderate hypothermia on the pharmacokinetics (PK) of amoxicillin in term neonates. Only few studies have been performed to evaluate the PK of this antibiotic in noncooled (pre)term neonates.

### WHAT QUESTION DID THIS STUDY ADDRESS?

☑ To our knowledge, this is the first prospective study evaluating and describing the PK of amoxicillin during all phases of controlled hypothermia in newborns, e.g., the hypothermic, rewarming, and normothermic phases.

### WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

☑ A description of the PK properties of amoxicillin in term newborns with hypoxic-ischemic encephalopathy (HIE) due to perinatal asphyxia receiving hypothermia treatment. Gestational age (GA), postnatal age (PNA), urine output, and body temperature were significant covariates on amoxicillin clearance (Cl).

### HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE

☑ Based on the newly developed PK-model, we advise an amoxicillin dosing regimen of 50 or 75 mg/kg/24h in three doses for patients with GA 36 or GA 38–42 weeks, respectively.

Term neonates who experience a severe hypoxic-ischemic insult during birth and develop encephalopathy are treated with hypothermia.<sup>1,2</sup> This improves long-term outcomes and reduces mortality rates.<sup>2–9</sup> These patients may suffer from early-onset sepsis with *Streptococcus agalactiae* (incidence rate 0.43% (95% confidence interval (CI) 0.37–0.49)), while *Listeria monocytogenes* should also be considered.<sup>10</sup> Because asphyxia due to infection is difficult to distinguish from asphyxia without infection, antibiotics, such as amoxicillin, are frequently used.<sup>11</sup> For efficacy, as a surrogate marker, the time that the nonprotein-bound concentration in

blood exceeds the minimum inhibitory concentration ( $T > MIC$ ) should be at least 40–50% in these patients.<sup>12</sup> Although  $\beta$ -lactam antibiotics such as amoxicillin are considered safe due to the wide therapeutic range, excessive accumulation can lead to the development of adverse drug events, such as seizures and crystalluria.<sup>13</sup>

Despite the widespread use of amoxicillin in (pre)term neonates, the literature describing its pharmacokinetic (PK) properties in these patients is sparse and mostly concerns small patient numbers or preterm infants,<sup>14–16</sup> while studies in hypothermic neonates are lacking altogether. As hypothermic treatment can

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**Table 1** Demographic data and samples drawn

Parameter	Amoxicillin total population ( <i>N</i> = 125) <sup>a</sup>	Amoxicillin index dataset ( <i>N</i> = 80) <sup>a</sup>	Amoxicillin validation dataset ( <i>N</i> = 45) <sup>a</sup>
GA (weeks) <sup>b</sup>	40 (36-42)	40 (36-42)	40 (37-42)
BW (grams) <sup>b</sup>	3340 (2090-5070)	3400 (2090-4835)	3310 (2295-5070)
Male	74 (59.2) <sup>c</sup>	49 (61.3) <sup>c</sup>	25 (56) <sup>c</sup>
PNA (days) <sup>d</sup>	5 (2-5)	5 (2-5)	5 (2-5)
SCr (μmol/L) <sup>e,f</sup>	55 (25-239)	53 (25-239)	57 (25-183)
Urine output (ml/kg/h) <sup>e,f</sup>	2.34 (0.04-10.00)	2.33 (0.04-5.40)	2.40 (0.10-10.00)
ASAT (U/L) <sup>e,f</sup>	83 (19-9179)	83 (22-1860)	84 (19-9719)
ALAT (U/L) <sup>e,f</sup>	38 (3-2631)	38 (3-518)	39 (5-2631)
MOF <sup>e</sup>	80 (64) <sup>c</sup>	53 (67) <sup>c</sup>	27 (60) <sup>c</sup>
Inotropic medication <sup>e</sup>	78 (62) <sup>c</sup>	51 (63.8) <sup>c</sup>	27 (60) <sup>c</sup>
Thompson score <sup>b</sup>	9 (3-19)	9 (3-19)	9 (3-19)
Samples total ( <i>N</i> ) during study	1280	841	439
Samples ( <i>N</i> ) per patient during study	10 (1-16)	10 (1-16)	11 (1-15)
Samples total ( <i>N</i> ) during hypothermic phase	882	575	307
Samples ( <i>N</i> ) per patient during hypothermic phase	8 (1-9)	8 (1-9)	8 (1-9)
Samples ( <i>N</i> ) during rewarming phase <sup>g</sup>	124	78	46
Samples total ( <i>N</i> ) during normothermic phase	274	188	86
Samples ( <i>N</i> ) per patient during normothermic phase	3 (1-6)	4 (1-6)	3 (1-6)

<sup>a</sup>Data are presented as median (minimum-maximum) unless stated otherwise. <sup>b</sup>Parameter measured at admittance. <sup>c</sup>Data presented as *n* (%). <sup>d</sup>Parameter measured at end of study period. <sup>e</sup>Parameter measured throughout study period. <sup>f</sup>Normal values for neonates PNA < 7 days: urine output: 1-3 ml/kg/h, SCr: 35-80 μmol/L, ASAT: <125 U/L and ALAT: <65U/L. <sup>g</sup>During rewarming samples were available as residual material of other laboratory monitoring. GA, gestational age; BW, birth weight; PNA, postnatal age; SCr, serum creatinine; MOF, multiorgan failure; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.

alter the PK of certain drugs,<sup>17</sup> studying its potential effect on amoxicillin is important.

We present the results of a *prospective* observational cohort study in asphyxiated newborns treated with moderate hypothermia and describe the population PK of amoxicillin in order to, if necessary, propose a more rational dosing regimen.

## RESULTS

### Patients

In total, 125 out of 187 newborns included in the PharmaCool Study<sup>11</sup> received amoxicillin during moderate hypothermia. Demographic and clinical characteristics, and samples drawn of the total, index-, and validation datasets are presented in **Table 1**. Twenty-seven (22%) patients died during the study period. However, in all cases this was not due to a persistent uncontrollable infection, but to multiorgan failure (MOF) or withdrawal of medical intensive care treatment in patients with an adverse prognosis. In total, 23 samples (2%) were below the limit of quantification. In **Supplementary Figure 1** the observed concentrations vs. the time after dose are shown. No serious adverse drugs events were recorded.

### Pharmacokinetic model building

With the index dataset (*N* = 80) a two-compartment model parameterized in terms of clearance (Cl), volume of distribution of the central (Vc) and peripheral (Vp) compartment, and

intercompartmental clearance (Q) was preferred to a one- and three-compartment model. Parameters were normalized *a priori* to a body weight of 70 kg using the 3/4 rule.<sup>18</sup> The allometric exponents could not be estimated, as this resulted in unstable models (nonconvergence in the iterative process), probably due to the small range of weight in the population.

Residual variability of the log-transformed data was described with an additive error model. Interindividual variability (IIV) could be estimated for Cl and Vc and was correlated (*r* = 0.49). Although the objective function value (OFV) decreased significantly by adding interoccasion variability (IOV), η-shrinkage was ±40%, reducing the power of IPRED and IWRES plots to detect model and residual model misspecification, respectively. Also, models were unstable (eigen values (EV) >1,000). Therefore, IOV was not included in the model. The structural model had an OFV of −675.6.

In the univariate covariate analysis postnatal age (PNA), body temperature (TEMP), urine output (UO), gestational age (GA), and inotropic comedication were identified as significant covariates on Cl, with ΔOFV of −435.1, −402.2, −163.4, −26.0, and −8.8 points, respectively. Postmenstrual age (PMA) also was a significant covariate on Cl (ΔOFV −266.9). However, as PNA alone gave a higher drop in OFV than PMA, PNA and GA were implemented as separate covariates on Cl (*P* < 0.001). No relationship

**Table 2** Parameter estimation of final pharmacokinetic models with final model associated bootstraps

Parameters	Model-building dataset (N = 80)					Total dataset (N = 125)				
	Final model		Final bootstrap			Final model		Final bootstrap		
	Estimates	CV (%)	Estimates <sup>#</sup>	CI (2.5%)	CI (97.5%)	Estimates	CV (%)	Estimates <sup>#</sup>	CI (2.5%)	CI (97.5%)
OFV	-1229.3	–	–	–	–	-1814.0	–	–	–	–
Cl (L h <sup>-1</sup> /70 kg)	3.03	5	3.06	2.74	3.35	2.92	4	2.94	2.71	3.21
Θ <sub>CL_PNA</sub>	0.20	19	0.21	0.13	0.28	0.22	12	0.23	0.16	0.29
Θ <sub>CL_TEMP</sub>	2.70	13	2.66	1.82	3.42	2.43	13	2.36	1.65	3.01
Θ <sub>CL_ URINE OUTPUT</sub>	0.11	13	0.11	0.3	0.19	0.08	6	0.08	0.02	0.16
Θ <sub>CL_GA</sub>	5.12	19	5.08	3.32	7.17	3.86	21	3.96	2.18	5.60
Vc (L/70 kg)	27.4	15	28.5	19.7	38.3	24.1	4	27.2	20.3	34.4
Q (L h <sup>-1</sup> /70kg)	8.8	14	8.30	6.30	11.37	7.93	12	7.46	5.61	9.70
Vp (L/70 kg)	24.0	9	23.3	19.2	27.8	24.1	4	23.1	20.3	26.1
Additive error	0.19	6	0.19	0.17	0.22	0.20	5	0.19	0.17	0.22
IIV Cl (%)	42.1	10	41.1	32.6	52.6	41.9	9	41.6	34.1	49.8
IIV Vc (%)	108.7	14	99.1	67.7	159.5	114.6	11	106.2	77.1	153.5
IIV additive residual error (%)	49.2	13	49.4	35.6	65.0	46.8	10	47.1	36.3	58.8

Final model:

$$TVCl = 2.92 \times (BW/70)^{0.75} \times (PNA/2.35)^{0.22} \times (TEMP/33.5)^{2.43} \times (URINE\ OUTPUT/2.99)^{0.08} \times (GA/280)^{3.86}$$

$$TVVc = 24.1 \times (BW/70)^1$$

$$TVQ = 7.93 \times (BW/70)^{0.75}$$

$$TVVp = 24.1 \times (BW/70)^1$$

#Median estimates. OFV, Objective Function Value; Cl, clearance; PNA, postnatal age (days); TEMP, body temperature (°C); URINE OUTPUT, daily urine output (ml/kg/h); GA, gestational age (days); CV, coefficient of variation; CI, confidence interval; Vc, volume of distribution of the central compartment; Q, intercompartmental clearance; Vp, volume of distribution of the peripheral compartment; IIV, interindividual variability; TV, typical value; BW, birth weight.

between Cl and gender, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), serum creatinine (SCr), urea, 1-h postnatal blood gas pH, Thompson score, or MOF was detected. Maturation models as covariates on Cl did not improve the PK model. Univariate analysis indicated that SCr, ASAT, and Thompson score were significant covariates for Vc, resulting in a drop in OFV of 38.8, 38.8, and 4.2 points, respectively. After the forward inclusion and backward deletion inotropic comedication on Cl and the covariates on Vc were discarded, while the other above-mentioned significant covariates were retained. The  $\eta$ - and  $\epsilon$ -shrinkage were <20%, rendering the EBE-based diagnostics useful for model evaluation.<sup>19</sup> Final parameter estimates are shown in **Table 2**. For a typical patient with GA 40 weeks, birthweight (BW) 3,000 g, 2 days PNA (i.e., TEMP 33.5°C), and normal UO (2 ml/kg/h), Cl increased from 0.26 L/h to 0.41 L/h at PNA 5 days and TEMP 37.0°C. The increase of PNA and TEMP contributed 23% and 27%, respectively, to the rise of Cl. Cl was 81% higher in patients with GA 42 weeks when compared to GA 36 weeks. An increase of UO from 0.5 to 6 ml/kg/h augmented Cl by 22%.

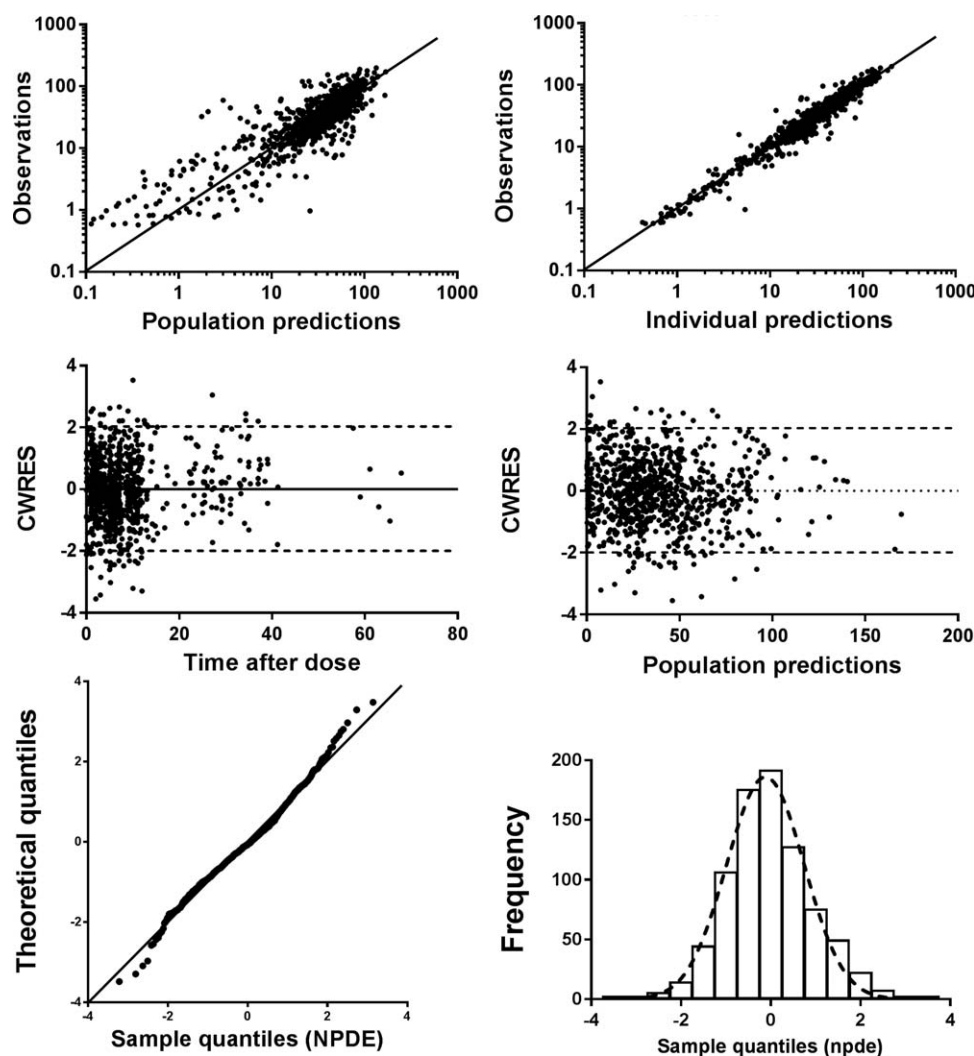
### Model evaluation and validation

**Table 2** gives the results of the bootstrap analysis of the final model developed with the index dataset (N = 80). As the results were in agreement with those of the final model developed with

the index dataset, the final model estimates are considered reliable. The goodness-of-fit (GOF) plots and normalized prediction distribution error (NPDE) plots are shown in **Figure 1**. These indicate that there was no major bias in the population component of the final model and that an appropriate structural model was found for most individuals. No ill-conditioning was found in the final model (EV 160). The IIV of Cl and CWRES vs. covariates and visual predictive checks (VPC) plots can be found in **Supplementary Figures 2 and 3**.

The results of the mean prediction error (MPE) and root mean squared prediction error (RMSE) calculated with the validation dataset (N = 45) are shown in **Table 3**. The bias and imprecision for concentrations <100 mg/L was acceptable. In **Supplementary Figure 4** the GOF and NPDE plots of the validation of the PK model built with the index dataset are shown.

The population PK parameters of the final model built with the total dataset (N = 125) were comparable to those of the final model built with the index dataset (**Table 2**). The results of the bootstrap analysis were in agreement with those of the final model developed with the total dataset. Variability between Cl and Vc was correlated with  $r = 0.48$ . The  $\eta$ - and  $\epsilon$ -shrinkage were <20%. No ill-conditioning was found in the final model (EV 446). In **Supplementary Figures 3 and 5** the GOF, NPDE and VPC plots are shown.



**Figure 1** Upper panel: Goodness-of-fit plots of observations (mg/L) vs. the population predicted (left) and individual predicted (right) concentrations (unit: mg/L). Middle panel: Conditional weighted residuals (CWRES) vs. time after dose (left; unit: h) and population predicted concentrations (right) of the final model built with index dataset ( $n = 80$ ). The solid line represents the line of identity. Lower panel: quantile-quantile (QQ)-plot of the metrics based on observations (black dots) vs. the theoretical  $N(0,1)$  distribution (dashed line) (left) and histogram of the normalized prediction distribution error (NPDE) distribution vs. the theoretical  $N(0,1)$  distribution (dashed line) (right).

### Simulations

The results of the simulations of 100 mg/kg/24h in three doses and 75 mg/kg/24h in three doses for patients with GA 36–42 weeks stratified for UO are presented in **Figure 2**. The first dosage regimen can result in very high concentrations, whereas the second dosage regimen will suffice for patients with GA  $\geq 38$  weeks irrespective of UO or TEMP as the  $T > MIC$  (1 mg/L) is 100% in all cases, while

there are no toxic ( $> 140$  mg/L) levels. However, taking into account the underprediction of the PK model at concentrations  $> 100$  mg/L, this regimen might lead to too high concentrations in patients with GA 36 weeks. For this group a regimen of 50 mg/kg/24h in three doses (**Figure 2**) will lead to adequate concentrations. In **Supplementary Table 1** the simulated trough and peak concentrations of these and other dosage regimens are displayed.

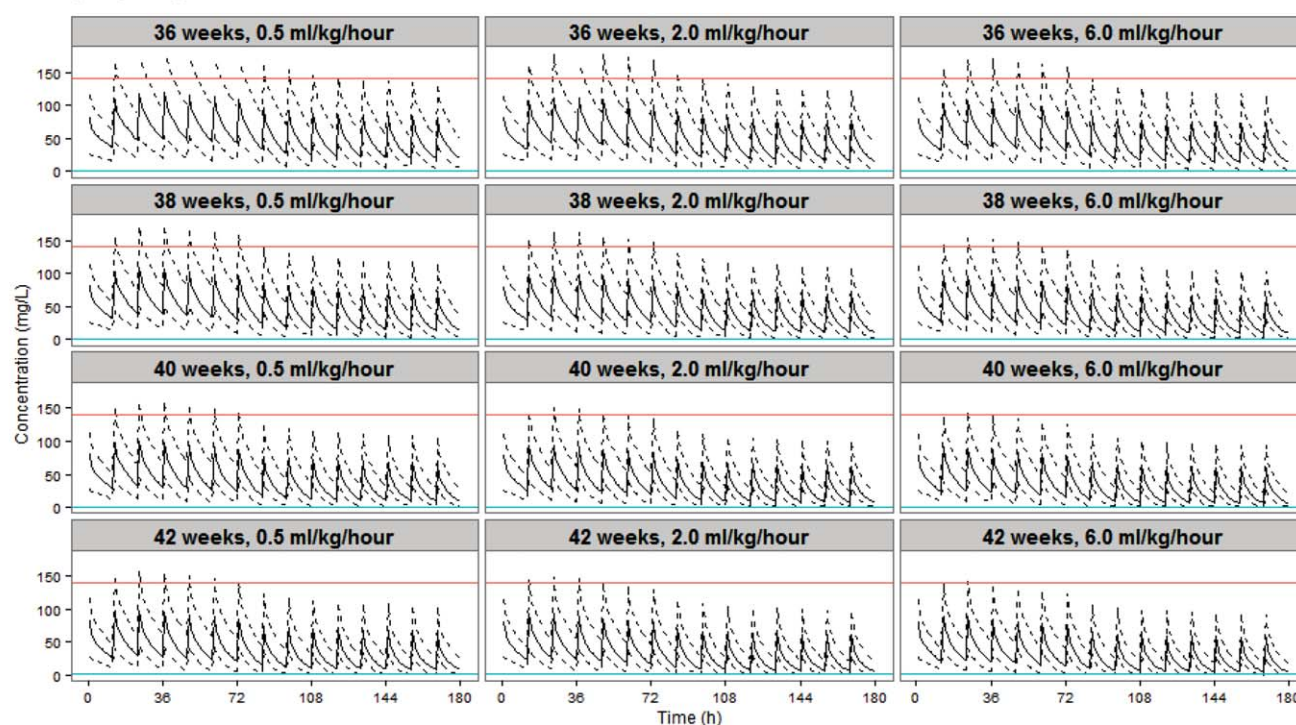
**Table 3** Predictive performance of the final model

	All concentrations ( $N = 439$ )	Concentrations $> 100$ mg/l ( $N = 29$ )	Concentrations $> 50$ – $100$ mg/l ( $N = 101$ )	Concentrations $> 10$ – $50$ mg/l ( $N = 230$ )	Concentrations $< 10$ mg/l ( $N = 79$ )
Bias (mg/L) (95% CI)	–5.3 (–7.5 to –3.0)	–56.6 (–73.5 to –39.8)	–13.0 (–17.2 to –8.7)	2.2 (0.3–4.0)	1.8 (0.6–3.0)
Imprecision (mg/L <sup>2</sup> ) (95% CI)	24.7 (19.9–28.7)	72.6 (50.8–89.2)	25.5 (22.1–28.5)	14.3 (12.5–16.0)	5.5 (2.1–7.5)

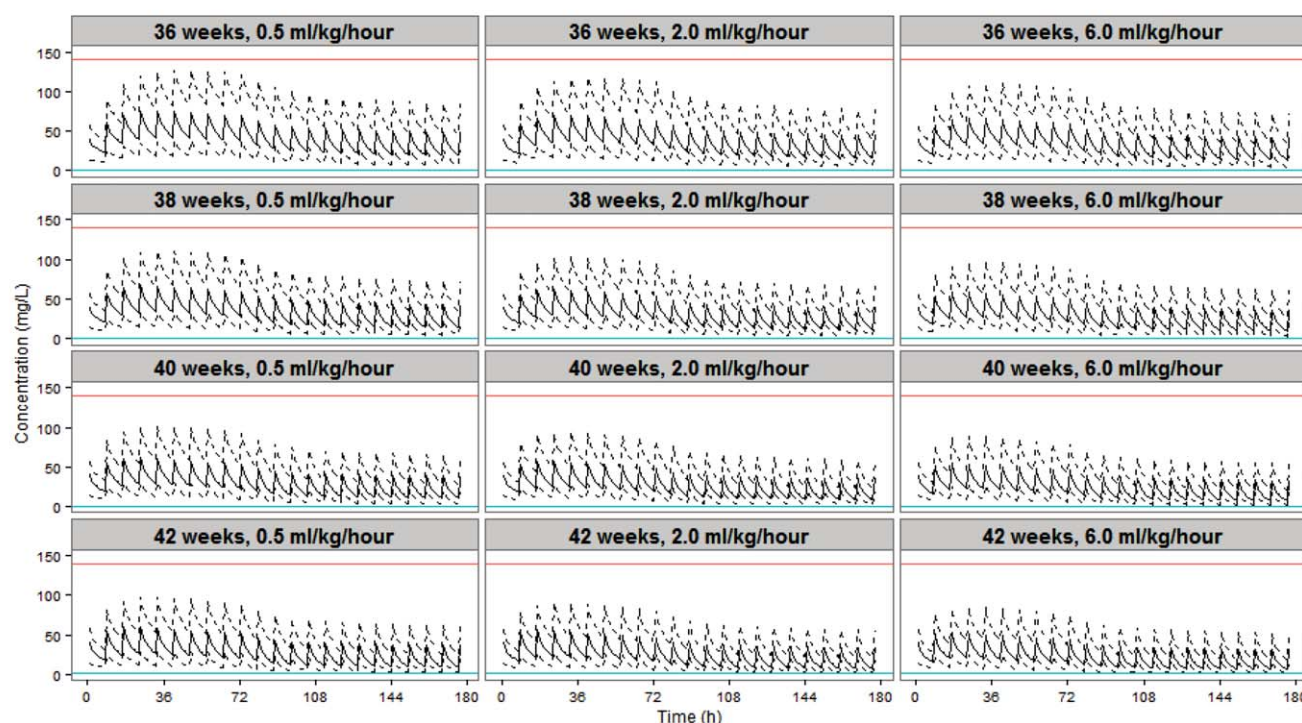
Bias, mean prediction error (MPE); Imprecision, root mean squared prediction error (RMSE). Larger values of RMSE indicate larger imprecision.



## 100 mg/kg/day in 2 doses



## 75 mg/kg/day in 3 doses



**Figure 2** Model-based predicted median concentration–time profiles (solid line) and the 5<sup>th</sup> and 95<sup>th</sup> percentile intervals (dashed lines) for patients with varying gestational ages (GA), body weights (BW) (GA 36 weeks (BW 2,260 g), 38 weeks (BW 2,380 g), 40 weeks (BW 2,950 g), and 42 weeks (BW 4,720 g), respectively), and varying urine output (UO) (0.5, 2.0 and 6.0 ml/kg/h) receiving 50 mg/kg every 12 h and 25 mg/kg amoxicillin every 8 h. For patients with GA of 36 weeks dosages of 50 mg/kg in four doses and 50 mg/kg in three doses are also shown. The solid lines at 1 and 140 mg/L depict the minimum and maximum target levels. The periods from 0–72 h, 72–96 h, and >96 h reflect the hypothermic, the rewarming, and the normothermic phase, respectively. [Color figure can be viewed at [cpt-journal.com](http://cpt-journal.com)]

## 50 mg/kg/day in 3 doses

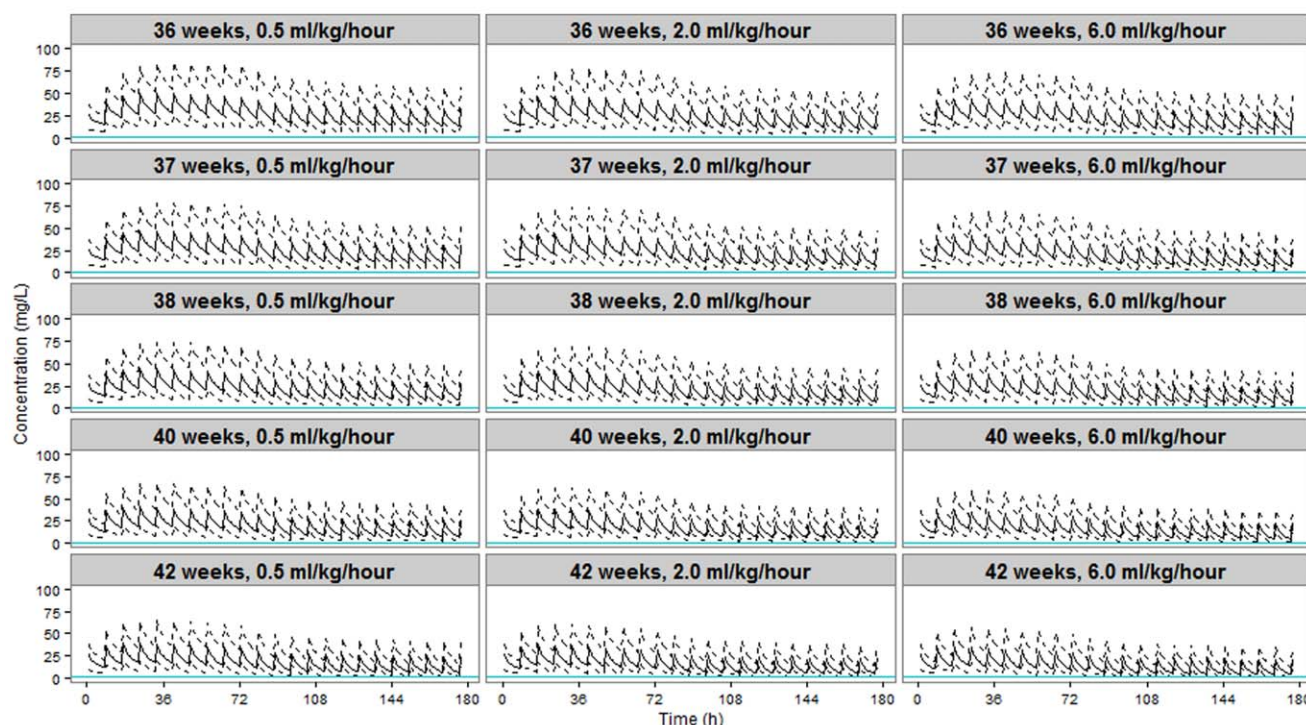


Figure 2 Continued.

## DISCUSSION

To our knowledge this is the first *prospective* study evaluating the PK characteristics of amoxicillin in a cohort of asphyxiated term infants with hypoxic ischemic encephalopathy (HIE) undergoing moderate hypothermia.

The PK properties of amoxicillin were described by a two-compartment model with *a priori* BW based allometric scaling and PNA, TEMP, GA, and UO being factors of influence on Cl. For a typical patient (GA 40 weeks, BW 3,000 g, 2 days PNA (TEMP 33.5°C), and normal UO (2 ml/kg/h)) Cl was 0.26 L/h (IIV 41.9%) increasing to 0.41 l/h at 5 days PNA (TEMP 37°C); the corresponding values expressed per kg weight (linear) are 0.09 and 0.14 L/h/kg, respectively. Two studies on the PK of amoxicillin in nonhypothermic term neonates found a Cl of 0.10 L/kg/h, which is similar to our findings.<sup>14,20</sup> If the typical patient was polyuric (UO 6 ml/kg/h), Cl increased by 22% compared to oliguria (UO 0.5 ml/kg/h). This is not surprising as, although in adults amoxicillin is mainly excreted by glomerular filtration and tubular excretion,<sup>21,22</sup> in neonates tubular secretion is very inefficient and glomerular filtration is believed to be the major pathway for elimination.<sup>15,23</sup> Furthermore, Cl increased with increasing GA and BW. PNA and GA are known predictors of glomerular filtration rate (GFR) maturation and drug Cl.<sup>15,24,25</sup> TEMP being an independent covariate is very interesting. It can be debated that PNA (reflecting GFR maturation and drug Cl) and TEMP are correlated and a distinction in the effect of both on Cl is not possible. However, a relationship in time is lacking (PNA increases linearly, while TEMP is constant during the first 72 h, where after it gradually increases to normothermia). Also,

the individual effects on Cl were significant (27% increase if TEMP rises from 33.5–37.0°C and 23% increase from PNA 2–5 days). A meta-analysis of several randomized controlled trials studying the effect of moderate hypothermia in neonates showed no significant difference in the incidence of renal impairment during cooling compared with standard NICU care.<sup>7</sup> Nevertheless, animal studies demonstrated that systemic mild hypothermia may reduce the GFR in the immature kidney.<sup>26</sup> Furthermore, in a previous study we found that cooling delayed normalization of gentamicin Cl, an antibiotic also mainly eliminated by glomerular filtration.<sup>27</sup>

For Vc no significant covariate-parameters relationship was identified besides BW. In our patient population the Vc was 0.34 L/kg (IIV of 114.6%) for a typical patient (GA 40 weeks, BW 3,000 g). The Vd of amoxicillin is increased in neonates, as this drug is water-soluble and thus mainly distributed in the relatively larger extracellular volume compared to adults.<sup>28</sup> In non-asphyxiated (pre)term neonates values of (0.41–0.68 l/kg) have been found.<sup>12,14,20</sup> However, these patients were not comparable to our population due to lower GA or larger PNA. A large IIV of V and Cl is also found in (pre)term nonasphyxiated neonates.<sup>15,16,20,25,29</sup>

The predictive performance of the final model developed with the index dataset showed a small statistically significant bias of −5.3 mg/L amoxicillin (imprecision 24.7 mg/L<sup>2</sup> (Table 3)). This is mainly due to the large bias in concentrations of >100 mg/L (−56.6 mg/L and 72.6 mg/L<sup>2</sup>, respectively). In the index dataset only 53 samples (6.3%) had a concentration >100 mg/L, which could explain this lower performance. Notwithstanding, the



NPDE plots showed that the model performed well in describing the individual data of the validation dataset. The underprediction of concentrations >100 mg/L have been taken into account in the proposed dosing regimen. Simulations based on our final PK model demonstrated that 50 mg/kg/day and 75 mg/kg/day amoxicillin in three doses (GA 36–37 and 38–42 weeks, respectively) for 7 days results in adequate concentrations ( $T > \text{MIC}$  100%) in the case of infections with *Streptococcus agalactiae* and *Listeria monocytogenes* (MIC 0.25 mg/L and 1.0 mg/L, respectively), while avoiding nontoxic peak levels (<140 mg/L) regardless of variation in the significant covariates on Cl (GA, PNA, TEMP, UO)<sup>14,30,31</sup> This is due to the wide therapeutic range of amoxicillin. The proposed dosage regimen is comparable to the dosage regimen of 20 mg/kg every 8 h advised for patients with GA >34 weeks by Pullen *et al.*<sup>14</sup> based on their PK analysis and simulations of amoxicillin in nonasphyxiated term neonates. They, however, maintained a higher target for effectiveness due to the MIC of 8 mg/L for *Enterococcus* species and species belonging to the enterobacteriaceae as causative agents of bacteremia.

As can be seen from the simulated trough levels (Figure 2 and Supplementary Table 1) of lower amoxicillin dosage regimens (e.g., 12.5 mg/kg/day in two doses) than the ones proposed would result in adequate concentrations. It is likely that lower doses may provide an optimal balance between efficacy and safety. However, finding the lowest dose for these patients was not an aim of this study. We have merely evaluated the current practice and described the PK of amoxicillin in these patients, as this information was lacking. If so desired, the effectivity of lower dosage regimens than the ones advised should be studied in prospective dose-finding studies.

We chose a maximum total concentration of 140 mg/L amoxicillin based on scarce literature of two case reports in adults receiving ampicillin i.v. with only one peak level and one mid-dose level measured.<sup>14,31</sup> Although a limited number of neurotoxic adverse events (including seizure, encephalopathy, and tremor) related to amoxicillin treatment have been reported, the role of epileptogenic drugs, such as penicillins, may be underestimated in neonates, as seizure detection in these patients is challenging.<sup>32</sup> It was striking that, despite the advice of the Dutch Pediatric Formulary (75 mg/kg/day amoxicillin in three doses in term neonates <1 week PNA and  $\geq 2,000$  g BW),<sup>33</sup> 62% patients received a median dose of 50 (range 28–100) mg/kg every 12 h, which could result in toxic peak levels, as shown by our simulations.

Due to ethical reasons it was not possible to prospectively compare our results with nonhypothermic HIE patients. In our opinion, comparison with a historic control group would not lead to a better evaluation, as the clinical characteristics of historic controls would not be comparable to our cohort due to many changes in intensive care treatment and admission criteria after the introduction of the hypothermia protocol. Also, an adequate historic control group is not available, as data were not collected as meticulously as in the current study before introduction of the hypothermia protocol.

We did not measure the free concentration of amoxicillin. Because the amount of protein binding of amoxicillin in neonates is low (about 10%),<sup>34</sup> this was considered negligible in determining our target concentration. Also, the effect of hypothermia on protein binding of amoxicillin was not evaluated. Although further investigation is warranted, this would be most beneficial in drugs that are highly protein-bound. The effect of a possible decrease of protein binding of amoxicillin is expected to have little clinical relevance.

Patients with GA <36 weeks were not included as, due to a lack of sufficient evidence, in the Netherlands and Belgium preterm infants are not treated with moderate hypothermia.

In conclusion, this study shows that the PK profile of amoxicillin in asphyxiated term neonates treated with moderate hypothermia was best described by a two-compartment model with PNA, TEMP, GA, and UO being primary factors of influence on Cl.

Based on simulations, we recommend 50 or 75 mg/kg/24h amoxicillin in three doses for patients with GA 36–37 or GA 38–42 weeks, respectively.

## METHODS

### Patients

Data were collected from a multicenter prospective observational cohort study conducted in 10 Dutch and 2 Belgian NICUs (the “PharmaCool Study,”<sup>11</sup> November 2010 until October 2014).

According to national protocol,<sup>35</sup> term newborns were cooled within 6 h after birth to a core TEMP of 33.5°C for 72 h if they met the criteria of perinatal asphyxia and ensuing encephalopathy.<sup>11</sup> Thereafter, patients were slowly (0.4°C/h) rewarmed to normothermia (37°C). All newborns undergoing moderate hypothermia were eligible for inclusion. Exclusion criteria were congenital hepatic or renal pathology, absence of central venous or arterial access for blood sampling, or lacking parental informed consent. One participating center also excluded patients with other serious congenital malformations or chromosomal abnormalities. The study was approved by the Institutional Review Board of each participating center.

### Data and sample collection

Data on GA, BW, gender, cause of the perinatal asphyxia, extent and duration of resuscitation, ventilator, and/or inotropic support, Thompson score, comedication, mean daily UO (ml/kg/h, collected via indwelling urine catheter), SCr, urea, ASAT, and ALAT were collected. Amoxicillin was prescribed in a dosage according to the local practice and administered as an i.v. bolus. One participating center administered amoxicillin/clavulanic acid as standard treatment. Dosing information and sampling times were recorded in a digital case report form. The dosage regimens are shown in Table 4. Despite the advised amoxicillin dosage published in the Dutch Pediatric Formulary,<sup>33</sup> the range of dosing regimens was large. As most infants were born in referring peripheral hospitals the information about the first amoxicillin dose was largely unknown. A standard dose of 25 mg/kg was presumed in those cases.

Blood samples for PK analysis of amoxicillin were collected: 6, 3, and 5 samples on days 2, 3 (hypothermia), and day 5 (normothermia), respectively.

The amoxicillin concentration was analyzed by zwitterion hydrophilic interaction chromatography coupled to tandem mass spectrometry (concentration range 0.5–40 mg/L) in only 10  $\mu$ L of plasma. The accuracy and imprecision at the lowest, middle, and upper limits of quantification were 105% and 10%, 102% and 6.5%, and 98% and <1.6%, respectively. Samples containing >40 mg/L amoxicillin were diluted 10 times and reanalyzed.



**Table 4** Observed dosage regimens of patients receiving amoxicillin (*N* = 125)

Total daily dose <sup>a</sup>	300 (60-740) mg / 91 (25-199) mg/kg
Duration of therapy <sup>a</sup>	4 (1-6) days
<i>N</i> (%)	Observed dosage regimen <sup>a</sup>
77 (61.6%)	50 (28-100) mg/kg b.i.d
41 (32.8%)	25 (23-28) mg/kg t.i.d.
1 (0.8%)	49 mg/kg q.i.d.
4 (3.2%)	30 (25-50) mg/kg single dose
1 (0.8%)	3 doses of 26 mg/kg q.i.d. followed by 26 mg/kg q.d.
1 (0.8%)	5 doses of 38 mg/kg b.i.d. followed by 27 mg/kg t.i.d.

<sup>a</sup>Median (range), unless stated otherwise. b.i.d., *bis in die*, two times a day; t.i.d., *ter in die*, three times a day; q.i.d., *quater in die*, four times a day; q.d., *quaque die*, once a day.

### Pharmacokinetic modeling

Data were analyzed using the first-order conditional estimation (FOCE) method with interaction option in the nonlinear mixed-effects modeling software NONMEM v. 7.2 (Globomax, Hanover, MD). Tools like R (<https://www.r-project.org/>, open-source, S-based statistical software, v. 0.98.945), XPose,<sup>36</sup> and PsN<sup>37</sup> were used to visualize and evaluate the models. The model-building process was performed in a stepwise fashion.

### Structural model

First, a random selection (every fourth patient upon inclusion) was made of 80 patients (the index dataset) from the total of 125 patients. The concentration data were log-transformed and one-, two-, and three-compartment models were fitted to the data. PK parameters were estimated as *Cl*, *V<sub>c</sub>*, *V<sub>p</sub>*, and *Q*. For all parameters IIV and covariance were tested assuming a log-normal distribution.<sup>38</sup> To account for variability in PK parameters due to the varying sizes of individual children, BW was included *a priori* in all model parameters using an allometric power model in which the parameter values were standardized to a body weight of 70 kg.

$$P = \theta_1 \times (BW/70)^{PWR} \quad (1)$$

where  $\theta_1$  is the typical value of the parameter *P* and *PWR* is the allometric scaling parameter, which was fixed at values of 0.75 and 1.0 for *Cl* and *V*, respectively.<sup>18</sup> *PWR* was also estimated to evaluate if this would result in a better fit. Residual variability was estimated using an additive error model. IOV was estimated with an occasion being a 24-h period. The likelihood ratio test was used to evaluate statistical significance between nested models where a reduction in the OFV  $\geq 3.9$  points was considered statistically significant ( $P < 0.05$  based on  $\chi^2$  distribution, *df* = 1). Also, GOF plots (PRED or IPRED vs. observations, individual conditional weighted residuals (iWRES) vs. IPRED, and CWRES vs. time), the total number of parameters, visual improvement of individual plots, correlation matrix, confidence intervals of parameter estimates, ill-conditioning, and  $\eta$ - and  $\epsilon$ -shrinkage were assessed.<sup>19,38</sup>

### Covariate model

Continuous covariates were tested separately in the model, using a power function equation:

$$P = \theta_1 \times (COV/COV_{\text{median}})^{\theta_2} \quad (2)$$

where  $\theta_1$  is the typical value of the parameter *P* in a patient with the median covariate value (*COV*) and  $\theta_2$  is the fractional change in *P* with

each unit of deviation from the median *COV*. Continuous covariates tested were PNA, PMA, GA, TEMP, ASAT, ALAT, SCr, urea, Thompson score, and UO. PMA was calculated by adding GA (in days) to PNA (in days). Categorical covariates were implemented in the model according to the following equation:

$$P = \theta_1 \times (\theta_2)^{COV} \quad (3)$$

where  $\theta_1$  is the typical value of the parameter *P* and  $\theta_2$  is the fractional difference in *P* between categories. For categorical dichotomous data (gender, MOF: yes/no, and inotropic comedication: yes/no) the value of the covariate was set to 0 for the reference classification and 1 for the other classification. MOF was considered to be present if a patient had renal or liver function failure as described by Shah et al.<sup>39</sup>

Maturation models (sigmoid *E<sub>max</sub>* functions and sigmoid hyperbolic functions) were tested to evaluate the effect of maturation of organ function on the parameter estimates.<sup>18,40</sup>

In a stepwise fashion the significance of covariates was tested.<sup>38</sup> In the forward inclusion a *P* value of  $< 0.05$  was applied (a decrease in OFV of at least 3.9 points, *df* = 1), while a more stringent *P* value of  $< 0.001$  was used in the backward deletion (a decrease in the OFV of at least 10.3 points, *df* = 1). To rule out bias across the covariates, plots of IIV and CWRES vs. the significant covariates were made.

### Model evaluation and simulations

**Model evaluation and validation.** To evaluate parameter precision and model stability a nonstratified nonparametric bootstrap analysis was performed using the PsN Toolkit<sup>37</sup> in which the index dataset was resampled 1,000 times to produce a new dataset the size of the original but with a different combination of individuals. The parameter estimates obtained with the bootstrap (median values and 95% CI) were compared to the parameter estimates of the final PK model.

The NPDE method was used to evaluate the predictive properties of the model. To do so, the index dataset was simulated 2,000 times in NONMEM using Monte-Carlo simulations in which the random effects were included. If the model adequately describes the simulated data, the NPDE is expected to follow a  $N(0,1)$  distribution. A histogram and quantile-quantile (QQ) plot of the NPDE distribution were used to evaluate the final model.<sup>41</sup> VPCs were performed to evaluate how well the final model describes the central tendency and variability of the observed data (simulation properties). The simulated median and 95% prediction intervals, derived from 1,000 datasets simulated from the developed model using the original dataset as a template, were compared to the ones from the observed data. The simulated and observed medians and 95% percentiles correspond closely if the model adequately describes the data.

The validation dataset contained the data of the remaining 45 patients. The population PK parameters obtained with the index dataset were fixed at the final parameters estimated and PRED were calculated using the same sampling times as measured in the validation dataset using the NONMEM MAXEVAL = 0 command.<sup>19,38</sup> Also, the GOF plots were evaluated. To evaluate the predictive performance of the final model the MPE (bias, Eq. 1) and RMSE (imprecision) were calculated for all concentrations.<sup>42</sup>

$$MPE = \frac{\sum_{j=1}^N (PE_j)}{N} \quad (4)$$

$$MSE = \frac{\sum_{j=1}^N (PE_j^2)}{N} \quad (5)$$

in which *N* is the number of pairs of true with predicted values and *PE<sub>j</sub>* is the relative prediction error for each pair (PRED-observed concentration). The RMSE is then calculated as the square root of the MSE

(mean squared error). The NPDE method was also used to evaluate the predictive properties of the final model developed with the index dataset.

Lastly, the model-building process, model evaluation, and internal validation were repeated as described above but with the data of all 125 patients that received amoxicillin (total dataset) to ensure that the same PK model would be developed as based on a random selection of the patients.

**Simulations.** To examine the optimal dosing regimen, Monte Carlo simulations ( $n = 1,000$ ) using the final model were performed in NONMEM on a selection of patients from the original database (GA 36 weeks (BW 2,260 g), 38 weeks (BW 2,380 g), 40 weeks (BW 2,950 g), and 42 weeks (BW 4,720 g)). The amoxicillin dosage regimens most frequently used in the patient population (100 mg/kg/24h in two doses and 75 mg/kg/24h in 3 doses) were simulated. For patients with GA 36 weeks a lower dosage regimen (50 mg/kg/24 h in three doses) was evaluated. Although in clinical practice UO can vary within patients, UO was fixed at 0.5 ml/kg/h (oliguria), 2 ml/kg/h (normal UO), or 6 ml/kg/h (polyuria) for graphical display. TEMP was set at 33.5°C during 0–72 h PNA, where after it increased with 0.4°C/h to normothermia (37°C). The median (including 5<sup>th</sup> and 95<sup>th</sup> percentiles) of the simulated concentrations ( $n = 1,000$ , samples drawn every hour) and simulated trough and peak concentrations (including interquartile range) were computed.

The MIC of value ranges of amoxicillin for *Streptococcus agalactiae* and *Listeria monocytogenes*, the most important neonatal pathogens causing early-onset sepsis in the Netherlands, are 0.25 mg/L and 1.0 mg/L, respectively.<sup>30</sup> As amoxicillin plasma protein binding in neonates is low (about 10%),<sup>34</sup> we assumed that the simulated amoxicillin concentrations must be above 1 mg/L for at least 40–50% of the dosing interval to ascertain bacteriologic efficacy.<sup>12</sup>

Additional Supporting Information may be found in the online version of this article.

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## CONFLICT OF INTEREST/DISCLOSURE

The authors declared no other conflict of interest.

## AUTHOR CONTRIBUTIONS

Y.A.B., T.R.d.H., and R.A.A.M. wrote the article; Y.A.B., T.R.d.H., D.H.G.M.N., A.H.v.K., R.A.A.M., J.H.v.d.L., F.G., and R.C.J.d.J. designed the research; T.R.d.H., D.H.G.M.N., A.H.v.K., J.H.v.d.L., F.G., P.H.D., A.v.H., S.H.P.S., K.P.D., H.L.M.v.S., M.R., I.A.Z., F.C., and A.Z. performed the research; Y.A.B., T.R.d.H., and R.A.A.M. analyzed the data; Y.A.B., T.R.d.H., and R.A.A.M. contributed new reagents/analytical tools.

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